Low rate of genotypic HIV-1 drug-resistant strains in the Senegalese government initiative of access to antiretroviral therapy

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Objective: To monitor the prevalence of antiretroviral (ARV)-resistant HIV-1 viruses, and the genotypic mutations in patients enrolled in the Senegalese initiative for access to antiretroviral treatment (ART).

Methods: A total of 80 patients with a virological follow-up of at least 6 months were selected, 68 were ART-naive and 12 ART-experienced. Genotypic resistance to ARV was studied at baseline for a random subset of patients and at each rebound in plasma viral load during ART, by sequencing the protease and reverse transcriptase genes.

Results: At baseline, 66 patients received highly active antiretroviral therapy (HAART) [2 nucleoside reverse transcriptase inhibitors (NRTIs) +1 protease inhibitor (PI) (n = 64) or 2 NRTIs + 1 non-nucleoside reverse transcriptase inhibitor (NNRTI) (n = 2)] and 14 patients (17.5%) started with a dual therapy because of ongoing anti-tubercular therapy or efficient previous bitherapy for the ART-experienced patients. The emergence of drug-resistant viruses (n = 13) during follow-up was more frequent in ART-experienced patients than in ART-naive patients, 41.7 versus 11.8%, resistant viruses emerged at comparable follow-up periods, a median of 17.8 and 18.3 months, respectively. In patients receiving zidovudine and lamivudine in their drug regimen, resistance to lamivudine was more frequent than to zidovudine. Two of the three patients, with viruses resistant to PIs, acquired mutations associated with cross-resistance. Strikingly, five (39%) of the 13 patients developed resistances to drugs that they had never received (n = 3) or that they received 18 or 36 months ago (n = 2). Didanosine/stavudine pressure had selected zidovudine-resistant viruses in four patients, and indinavir had selected a nelfinavir-resistant virus in one patient.

Conclusion: In contrast to other reports from developing countries where patients had received ARVs in an uncontrolled manner, our study showed that implementation of HAART together with good clinical, biological and logistical monitoring can reduce the emergence of resistant strains in Africa.

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Introduction

Highly active antiretroviral therapy (HAART) has greatly reduced HIV/AIDS-related morbidity and mortality in the industrialized countries. However, in sub-Saharan Africa, where more than 70% of all HIV-infected patients live, access to antiretroviral therapy is still restricted despite the strenuous efforts of governments, international institutions and pharmaceutical companies to reduce therapy costs. The need for relatively sophisticated laboratory facilities for treatment monitoring, and the infrastructure required to provide an uninterrupted supply of drugs are additional limitations on widespread use of HAART in poor countries.

Antiretroviral drugs (ARV) have been designed, tested and validated against the European and North American subtype B strains, but non-B subtypes predominate worldwide, notably in Africa. The efficiency of antiretroviral treatment (ART) can be influenced by the viral diversity. Like HIV-2, HIV-1 group O viruses are naturally resistant to non-nucleoside reverse transcriptase inhibitors (NNRTIs) [1]. Within group M, some subtype F samples are less susceptible to the tetrahydroimidazo (4,5,1-jk) (1,4)-benzodiazepin-2-(1H)-one and -thione (TIBO) derivate, a NNRTI [2], and subtype G strains have decreased *in vitro* susceptibility to protease inhibitors (PIs) [3]. Among women receiving single-dose nevirapine prophylaxis to prevent mother-to-child transmission, subtype D viruses may develop resistance to nevirapine more rapidly than subtype A [4]. Many amino acid mutations associated with minor resistance to PIs have been reported as natural variants in treatment-naive patients infected with non-subtype B HIV-1 strains [5–7], but their biological consequences remain to be studied.

One of the first antiretroviral therapy initiatives to be sponsored by an African government was launched in Senegal, in August 1998. This initiative provided the opportunity to examine certain key operational questions concerning the use of HAART in the African context. In this cohort, clinical and biological results in ART-naive patients were comparable with those seen in western cohorts, despite differences in the HIV-1 subtype distribution and an advanced disease stage when the treatment was initiated [8]. Replication of HIV-1 with drug-resistant viruses during combination therapy is considered to be a major cause of treatment failure. Actually, no data are available on development of resistance to ARVs in Africa in a well-documented group of patients. In this study, we describe the prevalence and the genotypic mutations of ARV-resistant viruses in patients enrolled in the Senegalese initiative of access to ARVs.

Patients and methods

Patients

A total of 80 patients, enrolled in the Senegalese initiative of access to ARVs (ISAARV) between August 1998 and February 2001, with a virological follow-up of at least 6 months, were selected for this study. Among these 80 patients, 68 were ART-naive and 12 were ART-experienced at inclusion.

The consenting patients were eligible if they bore certain medical and social criteria as previously described [8]. Briefly, ART-naive patients were eligible if they were asymptomatic with CD4 cell counts below 350×10^6 cells/l and plasma HIV-1-RNA levels above 100 000 copies/ml, or mildly symptomatic with CD4 cell counts below 350×10^6 cells/l, or at clinical AIDS-stage. No such criteria were mandatory for patients with previous ART history. The patients were clinically monitored on a monthly basis in one of the three major hospitals in Dakar. Initially, the first line antiretroviral regimen was based on two nucleoside reverse transcriptase inhibitors (NRTIs) and one PI, except for mildly symptomatic patients with plasma HIV-1 RNA below 10 000 copies/ml who received only two NRTIs. Late in 2000, following the updated international recommendations from the International AIDS Society [9], HAART based on a combination of two NRTIs plus one PI or one NNRTI became the first line regimen for all patients. Four NRTIs [stavudine (d4T); didanosine (ddI); zidovudine (ZDV); and lamivudine (3TC)], one PI [indinavir (IDV)] and one NNRTI [nevirapine (NVP)] were available. Adverse effects were assessed using the WHO toxicity scale. Adherence was assessed on the basis of the patients' statements to the physicians at each monthly visit. It was calculated as the ratio between the number of respected doses and the number of prescribed doses. The national ethics committee on AIDS approved this study.

Plasma HIV-1-RNA assay and CD4 cell counts

Plasma HIV-1-RNA levels were initially determined using the Bayer branched DNA HIV-1 Quantiplex assay (Bayer Diagnostics, Emeryville, California, USA) version 2.0 (bDNA 2.0, measurement range 500 to 800 000 copies/ml), and subsequently with the ultrasensitive version 3.0 (bDNA 3.0, measurement range 50 to 500 000 copies/ml). Plasma samples were stored at –80°C until assay. CD4 cell counts were determined with a FACSCount apparatus (Becton Dickinson, Mountain View, California, USA) in freshly collected whole blood. Plasma HIV-1 RNA and CD4 cell values were done at baseline (J0), after one month of treatment (M1, plasma HIV-1 RNA only), at 6 months of treatment (M6) and subsequently every 6 months.

Table 1. Demographic and clinical baseline characteristics of the 80 patients by antiretroviral-experience groups (Dakar, Senegal, 1998–2001).

Characteristics	ART-naive patients (n = 68)		ART-experienced patients (n = 12)		*P*
Demography					
Sex – no. (%)					
Male	38	(55.9)	4	(33.3)	
Female	30	(44.1)	8	(66.7)	0.1
Median age (IQR[a]) (years)	42	(32–47)	38	(33–43)	0.4
Clinical data					
CDC class – no. (%)					
Class A	1	(1.5)	3	(25.0)	
Class B	20	(29.4)	4	(33.3)	
Class C	47	(69.1)	5	(41.7)	0.01
Median CD4 cell count x 10^6/l (IQR[a])	112	(34–217)	237	(148–354)	0.02
Median plasma HIV-1 RNA (IQR[a]), (copies/ml)	95740	(22170–225200)	1032	(662–53360)	< 0.001
Median body mass index (IQR[a])	20.6	(18.5–22.6)	23.5	(20.1–26.4)	0.01
Antiretroviral treatment – no. (%)					
2 NRTI	9	(13.2)	5	(41.7)	
2 NRTI + 1 PI	57	(83.8)	7	(58.3)	
2 NRTI + 1 NNRTI	2	(2.9)	0	–	0.07
Median length of follow-up (IQR[a]) (months)	18.4	(11.9–30.0)	30.0	(24.3–32.7)	0.04

[a] IQR, interquartile range. NRTI, nucleoside reverse transcriptase inhibitor; NNRTI non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor.

Genotypic resistance testing

Genotypic resistance to ARVs was studied by sequencing the protease and reverse transcriptase (RT) genes as previously described [5]. Briefly, the viral RNA was extracted from plasma with QIAamp Viral RNA mini kit (QIAgen, Courtaboeuf, France) and retrotranscribed to complementary DNA by using Expand RT (Boehringer Mannheim, Mannheim, Germany) with a reverse primer. A 1800-bp fragment encompassing the protease and RT genes was amplified by nested-polymerase chain reaction and was directly sequenced (ABIPRISM Big Dye Terminator cycle sequencing ready reaction kit, Applied Biosystem, Roissy, France). Genetic subtypes were determined with phylogenetic tree analysis, using the Clustal W program as previously described [5,10]. The deduced amino acid sequences were compared to a reference sequence to detect mutations associated with resistance. These mutations were classified into minor mutations and major mutations, according to the consensus statements on ARV resistance of the Stanford HIV RT and Protease Sequence database [11].

Genotypic resistance testing was done at baseline for a random subset of patients and at each rebound in plasma viral load during ART. Viral rebound was defined as detectable viral load above 1000 copies/ml, which is also the detection limit of the genotypic resistance test, after having been undetectable. Genotypic resistance testing was also carried out for patients with non-optimal virological response after at least 6 months of treatment, defined as viral load above 1000 copies/ml without having ever been undetectable

Statistical analysis

Data were analysed using EPI-INFO 6.04 (Centers for Disease Control and Prevention, Atlanta, Georgia, USA) and STATA Release 7.0 (STATA Corporation, College Station, Texas, USA) software. The chi-square test and Fisher's exact test, for small sample sizes, were used to compare the distribution of qualitative variables between ART-naive and ART-experienced patients. For continuous variables, comparisons were based on the non-parametric Mann–Whitney test. The same tests were used for analysis of factors associated with development of resistant viruses. For this analysis, the data for the patients concerned were censored at the moment that resistant viruses emerged. All statistical tests were interpreted at the 5% significance level and 95% confidence intervals (CI) calculated by the binomial exact method were computed for proportions.

Results

Baseline characteristics of the patients

Eighty patients, with a virological follow-up of at least 6 months, were selected for this study. Table 1 summarizes the patient characteristics at inclusion. Twelve

patients used ART before inclusion [median, 7.5 months; interquartile range (IQR), 2–18 months]. Overall, patients were predominantly of middle age (median, 40.5 years; IQR, 32.0–45.5 years), 52.5% were male and the majority were at an advanced stage of HIV disease (81.3% had AIDS). In comparison with ART-naive patients, those that had experienced ARV use had less advanced disease, had lower viral load and had higher CD4 cell counts.

Globally, antiretroviral therapy combined NRTIs with one PI or NNRTI. At baseline, in addition to indinavir, 46 patients (57.5%) were prescribed d4T and ddI; 11 (13.8%) ZDV and 3TC; three (3.8%) ZDV and ddI; three (3.8%) d4T and 3TC; and one (1.3%) ddI and 3TC. Only two patients (2.5%) received nevirapine (NVP), one with d4T and ddI, and one with ZDV and 3TC. Fourteen patients (17.5%) started with a dual therapy because of ongoing antitubercular therapy including rifampicin, efficient previous bitherapy in ART-experienced patients, or for mildly symptomatic patients with plasma HIV-1-RNA load below 10 000 copies/ml. Of them, 13 patients received d4T and ddI and one ZDV and 3TC. The median length of follow-up was 18.4 months for ART-naive patients and 30 months for ART-experienced patients. The longer follow-up of ART-experienced patients can be explained by the fact that the first patients included in the Senegalese initiative of access to ARV were those already receiving ART but having difficulties in continuing to pay for their treatment.

Genotyping at baseline in ART-naive and ART-experienced patients

At inclusion, only one of the 65 treatment-naive patients tested had an undetectable viral load (< 500 copies/ml) versus four of the 12 ART-experienced patients. As two of the treatment-naive patients were infected with an HIV-1 group O virus, undetectable by commercial viral load assays, we used an in-house semi-quantitative assay [12] to measure viral load in these patients; the plasma HIV-1-RNA levels ranged between 2×10^3–2×10^4 copies/ml and 2×10^4–2×10^5 copies/ml, respectively.

To determine whether drug resistance was present at baseline, we sequenced the protease and RT genes of specimens from 41 (60.3%) of the 68 ART-naive patients. Similarly, six of eight ART-experienced patients with detectable viral load were also genetically characterized to optimize their treatment, two patients could not be analysed because their viral load was below the detection limit of the genotypic resistance assay (< 1000 copies/ml).

Phylogenetic tree analysis of the 47 *pol* sequences revealed a high genetic diversity; CRF02 was predominant but multiple subtypes and other circulating recombinant forms (CRF) co-circulated: CRF02-AG (n = 25, 53.2%), A (n = 5, 10.6%), C (n = 5, 10.6%), B (n = 3, 6.4%), CRF06 (n = 3, 6.4%), G (n = 2, 4.2%), D (n = 1, 2.2%), a unique recombinant U/K (n = 1, 2.2%) and HIV-1 group O (n = 2, 4.2%).

Among the treatment-naive patients, no major mutations conferring resistance to NRTIs, NNRTIs and PIs were observed, except for the two HIV-1 group O strains which were, like all previously described group O viruses, also naturally resistant to NNRTIs (Y181C). Many minor mutations were observed in the protease gene: M36I (n = 37), K20M/R/I/V/C (n = 30), L63P/A/S/T/N (n = 19), L10I/V (n = 10), I93L (n = 4, all subtype C), V82I [n = 3, specific to subtype G (n = 2), and also for 1 subtype C], I93V (n = 2, all subtype G), D60K/N (n = 2, group O only), A71V (n = 2, group O only), V77I (n = 2), and K45R (n = 1). The RT gene was less polymorphic: R211K (n = 20), V179I/D/E (n = 7 including the two group O viruses), A98S (n = 2, subtype G), A98G (n = 2, group O), V118I (n = 1), K219N (n = 1) and G333E (n = 1).

Among the six ART-experienced patients, only one patient was resistant to ARV, more precisely to ZDV and possibly also to d4T and abacavir, related to the combination of the following mutations: M41L, D67N, L210W, and T215Y. This patient had been treated with ZDV and ddI, before baseline. Another patient, who had previously been treated with ddI, had selected a minor mutation, K65R, associated with a possible resistance to zalcitabine (ddc) and ddI. Similarly as in the treatment-naive population, many minor mutations were also observed in the protease gene (L10I, K20I, M36I, K45R, and L63P) and only a few in the RT gene (V179I, R211K, and G333E).

Viral load rebound and genotypic resistance during patient follow-up

The plasma HIV-1 RNA level fell markedly after treatment initiation and became undetectable (< 500 copies/ml) after 1 month in the majority (77.9%) of the patients. Genotypic resistance testing was carried out for each viral rebound (> 1000 copies/ml) observed during follow-up. The two group O patients were responding well to their therapy (ddI/d4T/IDV), viral load was below 1000 copies/ml at 30-month follow-up.

ART-naive patients

For 30 of the 68 patients, viral rebounds were observed after between 6 and 36 months of follow-up. Certain viral rebounds (n = 22) were associated with partial or total treatment interruption due to adverse effects or incompatibilities with treatment regimens for opportunistic infections, or to poor adherence. No resistance mutations were observed and viral load became again undetectable in these patients after reinstatement of HAART. The viral rebounds were associated with the

Table 2. Emergence of resistant viruses in antiretroviral therapy-naive and ART-experienced populations (Dakar, Senegal, 1998–2001).

Samples	Baseline (JO)/ viral rebound	Treatments	Mutations in the reverse transcriptase gene												Mutations in the protease gene									Selected resistances to following drugs	*pol* subtypes
			M41	K65	D67	K70	**V75**[d]	A98	118	**M184**	L210	R211	**T215**	K219	L10	K20	M36	L63	A71	G73	**V82**	**I84**	**N88**		
101HALD	JO	ZDV, 3TC, IDV																A							B
	M12									**V**								A						3TC	
34HALD	JO	ZDV, 3TC, IDV														I	I								CRF02-AG
	M24									**V**						I	I							3TC	
65HALD	JO	ddI, d4T[a]										K			V	I	I								CRF02-AG
	M2			R								K			V	I	I								
	M18	ZDV, 3TC, IDV								**V**		K			V	I	I	P						3TC	
69HPD	JO	ddI, d4T, IDV[b]															I								A
	M24	ZDV, 3TC, IDV	L						I	**V**			**Y**				I	P	V	S		**V**		3TC, PIs, (NRTIs)	
42HPD	JO	ddI, d4T[c]										K				I	I	N							CRF02-AG
	M18	ddI, d4T, IDV					**T**					K				I	I							d4T	
46HALD	JO	ddI, d4T, IDV										K				I	I								CRF02-AG
	M18					R						K		E		I	I							ZDV (intermediate)	
160HALD	JO	ddI, d4T, IDV										K					I	P							D
	M12											K					I	P	T				**D**	NFV	
63HPD	JO	ddI, d4T, IDV						S							I	I	I	I	P		I				G
	M30							S							I	I	I	I	P		**T**			IDV, RTV, NFV (AMP)	
20HALD	<JO-JO	ZDV, 3TC, IDV																						nt	
	M6									**V**					V									3TC	B
21HALD	<JO-JO	ZDV, 3TC, IDV																						nt	
	M18									**V**														3TC	B
74HALD	<JO-JO	ddI, d4T										K				I	I								
	M12											K	**Y**			I	I							ZDV	CRF02-AG
53HPD	<JO	ZDV, ddI																						nt	
	M18	ddI, d4T	L		N				I		W	K	**Y**			I	I							ZDV (d4T, ABC)	CRF02-AG
55HPD	<JO	ZDV, ddC																						nt	
	M36	ddI, d4T			N							K	**Y**	R			I							ZDV (d4T, ABC)	D

For each patient, previous (<JO= before ART initiation, for ART-experienced population) and actual treatment molecules and duration (in months) are indicated as well as genotyping results. nt (not treated) indicates no genotypic results because of undetectable viral load. [a]This patient interrupted his treatment between M5 and M7. [b]This patient interrupted PI between M4 and M6 (bi-therapy). [c]This patient received this treatment during 5 months. [d]The major mutations are noted in bold. Drugs used: IDV, indinavir; RTV, ritonavir; NFV, nelfinavir; AMP, amprenavir; PI, protease inhibitors; ZDV, zidovudine; 3TC, lamivudine; ddI, didanosine; ddC, zalcitabine; d4T, stavudine; ABC, abacavir.

emergence of resistant strains in only eight patients. The prevalence of resistant viruses in the 68 naive patients was thus 11.8% (CI, 5.2–21.9%). Table 2 summarizes the treatment regimens for the eight patients who developed resistant viruses and shows the selected mutations compared to baseline profiles if applicable. Resistant viruses appeared after between 12 and 30 months (median, 18.3 months) of therapy, with mutation profiles conferring resistance to: 3TC (n = 3), nelfinavir (n = 1), ZDV (n = 1), d4T (n = 1), NRTIs/3TC/PIs (n = 1), indinavir/ritonavir/nelfinavir (n = 1). Resistance to 3TC (n = 4) was frequently and rapidly selected after 10 to 24 months of exposure to 3TC. Cross-resistance to several PIs was observed in one of two subtype G samples, the genotypic profile in the protease gene is almost similar between baseline and 30 months of follow-up, except the substitution at position 82. The natural mutation V82I, a characteristic feature of subtype G, could be a mutation allowing a faster switch to the resistance mutation V82T.

Strikingly, two of the eight patients developed mutations associated with resistance to molecules that they had never received, after 12 and 18 months of tritherapy (IDV/ddI/d4T). One patient was resistant to nelfinavir (A71T, N88D) and one had an intermediary resistance to ZDV (K70R, K219E). For both patients, these mutations were absent at baseline.

ART-experienced patients

For six patients, viral rebounds were observed after between 6 and 36 months of ARV treatment. For one patient, this rebound was related to a treatment interruption and no resistant mutations were present. However, the other five patients developed resistances to 3TC (n = 2) and ZDV (n = 3) (Table 2). The prevalence of resistant viruses in the 12 ART-experienced patients was thus 41.7% (CI, 15.2–72.3%) and resistant viruses emerged after a median follow-up of 17.8 months. The M184V mutation conferring resistance to 3TC appeared rapidly, after between 6 and 18 months of treatment, but these patients had already received 3TC before baseline. Similar to the ART-naive patients, three patients developed mutations (T215Y) associated with ZDV resistance while they were not receiving ZDV. This mutation was selected after 12 months (74HALD), 18 months (53HPD) and 36 months (55HPD) of ddI/d4T treatment, only the two latter patients took ZDV before baseline. Overall, among the 12 ART-experienced patients, three of five (60%) patients receiving bitherapy (ddI/d4T) developed resistant strains, versus two of the seven (28.6%) patients receiving triple therapy.

Factors associated with emergence of ARV-resistant viruses

During follow-up, the resistant viruses appeared more frequently in ART-experienced patients than in ART-naive patients (41.7 versus 11.8%, $P = 0.02$). The length of follow-up in patients who developed resistance was similar or lower than for those who did not develop resistance, thus allowing comparison between groups. Although not significant, except for CD4 cell counts, ART-naive patients in whom resistant viruses were observed, seemed to be at a more advanced stage of HIV disease at baseline than those without resistance (Table 3). This trend was not found in ART-experienced patients but this group was too small. As expected, a temporary or permanent intake of bitherapy was more frequent in patients who developed resistance. The average monthly adherence was very similar in both groups as well as the adverse effects that can favour lower plasma concentration of drugs and adherence difficulties.

Discussion

Our results showed that among 80 patients, receiving ARV in the Senegalese initiative for access to ARV treatment, 13 (16.3%) harboured resistant viruses after a median follow-up of 24 months. The selection of resistant viruses was lower in the ART-naive population than in the ART-experienced population, 11.8 versus 41.7%. In both populations, resistant viruses emerged after comparable treatment duration, with a median of 18.3 and 17.8 months, respectively. The overall prevalence of resistant viruses was thus lower than in previous preliminary studies in Gabon and Ivory Coast, where more than 50% of patients receiving ARV, mainly as mono- or bi-therapy with limited or no biological monitoring for treatment efficiency, were resistant in less than 18 months of ARV use [13–15]. However it is important to note that the data on populations with uncontrolled ARV use are similar to those observed in our ART-experienced group, and more precisely to the group of patients receiving bitherapy only [13–15].

Many other viral rebounds (n = 23) were observed, but they were associated with treatment interruption for social or medical reasons or poor adherence.

In patients receiving ZDV and 3TC in their drug regimen, resistance to 3TC related to the M184V mutation was more frequent than resistance to ZDV conferred by the T215Y mutation, in accordance with another study in Uganda [16]. This could be in concordance with previous studies suggesting that the M184V mutation could have a protective effect on the emergence of ZDV-associated mutations [17]. Two of the three patients with viruses that were resistant to PIs acquired mutations associated with cross-resistance to PIs, which compromised the further use of PIs in these patients.

One of the most striking observations was that five (39%) of the 13 patients developed resistances to drugs that they never received (n = 3) or for which treatment was interrupted for 18 or 36 months (n = 2). The ddI/d4T pressure had selected ZDV-resistant viruses in

Table 3. Analysis of factors associated with occurrence of antiretroviral drug resistance in the antiretroviral therapy-naive and ART experienced patients (Dakar, Senegal, 1998–2001).

	ART-naive patients			ART-experienced patients		
Characteristics	ARV resistance (n = 8)	No ARV resistance (n = 60)	*P*	ARV resistance (n = 5)	No ARV resistance (n = 7)	*P*
Demography						
Sex (%)						
Male	62.5	55.0		60.0	14.3	
Female	37.5	45.0	1.0	40.0	85.7	0.2
Median age (IQR[a]) (years)	32 (29–49)	42 (32–46)	0.4	39 (33–44)	37 (32–43)	0.6
Baseline clinical data						
CDC class, (%)						
Class A	0.0	1.7		20.0	28.6	
Class B	12.5	31.7		60.0	14.3	
Class C	87.5	66.7	0.5	20.0	57.1	0.3
Median CD4 cell count x 10^6/l (IQR[a])	28 (7–92)	124 (55–228)	0.049	308 (223–344)	203 (62–472)	0.3
Median plasma HIV-1 RNA (IQR[a]),	108900	89515		947	1987	
(copies/ml)	(19560–155300)	(22845–235950)	0.9	(824–1000)	(500–89720)	0.4
Median body mass index (IQR[a])	19.8 (17.9–22.5)	20.6 (18.6v22.8)	0.8	23.6 (19.9–26.6)	23.4 (20.3–26.2)	0.8
Follow-up						
Median length of follow-up (IQR[a]), (months)	18.3 (16.4–23.1)	18.0 (11.7–30.0)	0.9	17.8 (12.4–19.7)	30.0 (6.0–30.1)	0.5
Lifetime bitherapy, (%)	37.5	26.7	0.7	60.0	28.6	0.6
Median average monthly adherence, (%)	96.5	96.0		99.7	99.7	
	(91.5–99.0)	(91.0–99.0)	0.7	(81.9–99.8)	(98.2–99.8)	0.7
Median number of adverse effects (IQR[a])	0 (0–1)	1 (0–1.5)	0.3	0 (0–1)	1(0–2)	0.2

[a] IQR, interquartile range.

four patients, and indinavir had selected nelfinavir-resistant virus in one patient. *In vivo* both ddI and d4T have the potential to select thymidine analog mutations (TAM: M41L, D67N, K70R, L210W, T215Y/F and K219Q/E) associated with ZDV resistance but this pressure is not as great as that exerted by ZDV. Viral isolates possessing such mutations have previously been described but with modest or no phenotypic resistance to ddI or d4T *in vitro* [18,19]. However, in our study, the appearance of these viruses was associated with an increase in viral load, thus suggesting phenotypic resistance. Phenotypic and clinical studies are needed to explain the mechanisms involved in the selection of resistant viruses to drugs that were not administered. It will also be important to find out whether this is more frequently observed in non-B HIV-1 strains.

In addition to the lack of ART efficacy related to resistant strains for treated patients, a real public health problem concerns the possible transmission of these resistant strains. In industrialized countries, 11% of new HIV-1 infections already have variants that are resistant to one or more ARVs and recent studies have shown that this continues to increase [20]. In Senegal, no ART-naive patients harboured resistant viruses at baseline, but ARVs were only recently introduced.

Non-B viruses have a higher prevalence of naturally occurring minor mutations, especially in the protease gene, which could lead to faster development of drug resistance than in B viruses. Frater *et al.* [21] studied the impact of baseline polymorphisms in RT and protease genes on the outcome of HAART in HIV-1-infected African patients, with a 1-year follow-up. The patients who were infected with non-B subtypes, notably subtypes A, C and D, responded efficiently to HAART. Similarly, in our study with a longer follow-up, numerous minor mutations in the protease gene did not seem to influence therapy outcome, except maybe for subtype G, which could develop resistance to PIs more rapidly, due to the pre-existing V82I mutation and many minor (n = 4) mutations in the protease. However, more long-term studies are necessary to determine the clinical significance of these mutations.

Our studies in Senegal show that implementation of HAART is possible in developing countries, and that tritherapy and good clinical, biological and logistical monitoring can reduce the emergence of resistant strains. With the recent efforts to lower the price of ARV, these drugs will now be massively introduced in many developing countries. In order to avoid the rapid emergence of resistant viruses on a large scale in the develop-

ing world, it is important that the infrastructures necessary to monitor ART are also rapidly implemented in these countries and that clinicians are trained in the appropriate use of ARV. There is a need for alternative, less sophisticated and cheaper tools to monitor CD4 cell counts and/or viral load closely. A continuous surveillance of the circulation of ARVs and ARV drug-resistant viruses has to be organized to guide ARV treatment strategies and policies.

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